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16. ABSTRACT

Nickel chloride was administered in drinking water to pregnant mice from the 2nd through the 17th day of gestation at nickel doses of 0, 500, or 1000 ppm. Fetal or maternal toxicity was not seen after administration of 500 ppm of nickel. However, the higher dose caused spontaneous abortions, loss of fetal mass in survivors, and loss of maternal mass. The oral route of administration via drinking water was at least 2.7 times less effective than parenteral routes in producing fetal effects.

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmnental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pokllutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for nonionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

The nickel-plating industry is the largest consumer of nickel. Nickel is also converted to an environmental pollutant when organic fuels are consumed. In these processes, there are always opportunities for the loss of nickel into the environment. It is, therefore, pertinent to determine the safety of these compounds for human health.

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ABSTRACT

Nickel chloride was administered in drinking water to pregnant mice from the 2nd through the 17th day of gestation at nickel doses of 0, 500, or 1000 ppm. Fetal or maternal toxicity was not seen after administration of 500 ppm of nickel. However, the higher dose caused spontaneous abortions, loss of fetal mass in survivors, and loss of maternal mass. The oral route of administration via drinking water was at least 2.7 times less effective than parenteral routes in producing fetal effects.

SECTION 1

CONCLUSIONS

The oral dosage of nickel (Ni) required to produce fetal toxicity or teratology may have been overestimated when based on studies using parenteral administration. A factor as large as 2.7 may be needed to correlate the effects of parenterally administered Ni with those of orally administered Ni.

SECTION 2

EXPERIMENTAL PROCEDURES

MATERIALS AND METHODS

Time-bred female mice (CD-1, Charles River Breeding Labs, Inc., Wilmington, Mass.) were used in these experiments. Mice were bred and shipped on the 1st day of gestation. (Presence of a copulatory plug is considered the 1st day of pregnancy.) Upon arrival, the mice were randomly assigned to sham or treatment groups in equal numbers, weighed, and housed in groups of four in shoe-box type cages. On the 18th day of pregnancy, each dam was killed by suffocation with CO_2 gas and weighed. The uterus was removed and counts were made of dead (resorbed and newly dead) and live fetuses. The live fetuses were removed, examined for gross abnormalities, blotted dry, and weighed. Two of every three fetuses were fixed in Bouin's solution, and the third fetus was preserved in alcohol.

In initial dose-finding experiments, nickel chloride ($NiCl_2$) water solutions were offered as drinking water ad lib to stock adult male CD-1 mice. The means of body weights over days were compared to those of similar sham mice on ordinary tap water. A 10,000-ppm Ni solution adjusted to a pH of 6 with phosphate buffer could not be used due to observable precipitation; however, acetate buffer sustained a 10,000-ppm solution at pH 6.2-6.5. When compared to tap-water controls, mice maintained with this solution as drinking water lost

26% of their body weight within 3 days (4 mice/group). Consumption of this Ni solution was insignificant (2% of body weight); shams consumed tap water equal to 16% of their mass in this same period. After a switch between the tap water and the Ni solution in these animals, the mice that were previously on tap water but were subsequently changed over to Ni solution lost 14% of their body weight in 1 day, while the mice that were switched to tap water almost regained their initial weights. Clearly, the 10,000-ppm Ni solution was not acceptable to mice as drinking water.

An additional attempt was made to induce consumption of the 10,000-ppm Ni solution by adding an artificial sweetener to water at concentrations of 1 and 10%, but water consumption was not increased, and severe loss of body weight ensued. However, mice did tolerate well a 1000-ppm Ni solution (pH 6-6.5) without sweetener, and, after an initial small but noticeable loss, the masses were approximately equal to initial size in 7 days. The 1000-ppm solution was then considered as the maximum tolerated dose in drinking water for this experiment.

Pilot groups of time-bred CD-1 mice were used to test the effects of gestational exposure to Ni solutions (pH 6-6.5) as drinking water. Tap or drinking water with Ni concentrations of 1000, 1500, 2000, 3000, or 5000 ppm were given to groups of 8 bred mice. The effects of dose on survival of the dam and incidence of pregnancy in survivors are shown in Table 1. The dose threshold for lethality of dams in this regimen was between 2000 and 3000 ppm. The threshold for total early lethality of the conceptuses resulting in a non-pregnant female was 1000 to 1500 ppm of Ni. Because a dose of 1000 ppm did

TABLE 1. SURVIVAL AND PREGNANCY RATES OF TIME-BRED CD-1 MICE GIVEN VARYING CONCENTRATIONS OF NI IN DRINKING WATER ON DAYS 2-17 OF GESTATION

Ni (ppm)	Survived (No.)	Pregnant Survivors (No.)	Pregnant Survivors (%)
1000	8/8	7/8	88
1500	8/8	4/8	50
2000	8/8	1/8	12
3000	4/8	0/4	0
5000	1/8	0/1	0

not appear to be lethal to either the pregnancy or the dam, our choice of this as the maximum tolerable dose appeared to be reasonable.

Since the mice were avoiding water with concentrations of Ni above 1000 ppm, to achieve voluntary consumption at this level we added $NiCl_2$ to the mouse feed (Bioserv, Frenchtown, NJ) and fed it to time-bred mice. All subjects avoided the feed containing 10,000-ppm Ni and showed severe loss of weight. The idea of attempting to force the consumption of Ni in the diet did not appear to be a successful strategy. The highest level of Ni we could attain was a concentration of 1000 ppm in water and a nominal 100 ppm in feed. Samples of the feed containing Ni in a nominal dose of 100 ppm were analyzed using neutron activation and found to contain 68 ± 5 μg Ni/g of feed.

The experiment to elicit the teratologic potential of Ni was begun using these concentrations. Time-bred mice were received from the supplier and

assigned to 3 groups of 24 to receive 1000-ppm Ni in water and 68 μg Ni/g of feed; or 7 groups of 12 to receive 500-ppm Ni in water and ordinary feed; and to equal groups of concurrent sham controls for each dose (to receive tap water and ordinary feed). When received, each group was allocated for handling as described earlier.

Analyses were conducted using analysis of variance techniques for each variable in each group of mice. Pregnancy rates and other incidental data were analyzed using contingency tables. Unless stated otherwise, all data were analyzed using the litter as the experimental unit.

SECTION 3

RESULTS

Rates of pregnancy in bred mice were affected when Ni was administered in drinking water at concentrations of 500 or 1000 ppm on days 2-17 of gestation. No statistical difference was observed in shams of this group; therefore, all shams were treated as a single 0-ppm group in Table 2. The ratio of non-pregnant to bred females in the shams of the 500-ppm group was 15:44 (34%) and the corresponding ratio in the shams of the 1000-ppm group was 9:31 (29%). A value of $P = 0.0001$ ($\chi^2 = 23.04$, $DF = 2$) resulted from pooling of shams and testing with contingency tables for differences in pregnancy rates across dose. The distinct decrease in the incidence of pregnancy in the group that received 1000 ppm (21% pregnant), when compared to the more normal rate of 65-68% seen in bred mice that received 0 or 500 ppm, was the obvious reason for this statistical result.

The body mass measurements in bred, non-pregnant mice are shown in Table 3. Analysis of these values revealed no statistical difference between sham animals used as concurrent controls (0-ppm Ni) and those receiving 500- or 1000-ppm Ni ($F = 1.73$, $DF = 1$, $P = 0.19$). However, when the sham values were pooled ($\bar{X} = 29.2$, $SD = 2$) and the analysis done on a dose basis (0, 500, or 1000 ppm), a significant decrease was seen in the body masses of bred but non-pregnant mice ($F = 4.90$, $DF = 2$, $P = 0.011$). Therefore, it appears that

the decrease in their mean body mass was caused by the consumption of drinking water dosed with 1000-ppm Ni but not by the dose level of 500 ppm.

TABLE 2. PREGNANCY INCIDENCES IN TIME-BRED CD-1 MICE GIVEN 0-, 500-, OR 1000-ppm NI IN DRINKING WATER ON DAYS 2-17 OF GESTATION

Ni (ppm)	Not Pregnant (No.)	Pregnant (No.)	Pregnant (%)
0	24	51	68
500	14	26	65
1000	27	7	21

TABLE 3. BODY MASSES (g) OF BRED BUT NON-PREGNANT CD-1 MICE GIVEN 0-, 500-, OR 1000-ppm NI IN DRINKING WATER ON DAYS 2-17 OF PRESUMED GESTATION

	Ni (ppm)	
	500	1000
Concurrent shams (0 ppm)	29.6 ± 1.9	28.6 ± 2.1
	N = 15	N = 9
Treated (500 or 1000 ppm)	29.7 ± 1.5	26.5 ± 1.7
	N = 14	N = 27

Table 4 contains the results of counts of fetuses and fetal masses in dams that received 0-, 500-, or 1000- ppm Ni in drinking water. The concurrent shams of each dose (500 or 1000 ppm) were pooled based on the lack of a statistical difference. The numbers of living, dead, or total fetuses in litters were not significantly different in dams receiving 0, 500, or 1000 ppm. Fetal mass per litter was significantly decreased in litters receiving 1000-ppm Ni ($P = .007$, $F = 5.37$, $DF = 2$).

TABLE 4. NUMBERS OF FETUSES OF PREGNANT CD-1 MICE GIVEN 0-, 500-, OR 1000-ppm NI IN DRINKING WATER ON DAYS 2-17 OF GESTATION

	Ni (ppm)		
	0	500	1000
Litters (No.)	51	26	7
Live fetuses*	10.4 (2.6)	11.6 (2.0)	11.7 (3.3)
Dead or resorbed fetuses*	0.78 (0.92)	0.46 (0.58)	0.86 (0.90)
Total fetuses*	11.2 (2.5)	12.0 (1.8)	12.6 (2.7)
Fetal mass (g)*	1.00 (0.12)	0.99 (0.07)	0.86 (0.09)

*Means and SD (numbers in parentheses).

SECTION 4

DISCUSSION

The rate of water consumption in mice was 160 ml/kg body mass/24 h estimated by observations of bred mice gang-caged from the 14th through the 18th day of gestation. During 24 h of this study, the consumption of Ni at concentrations of 500 and 1000 ppm in water was estimated to be 80 and 160 mg/kg body mass, respectively. For the period of administration (2nd through 17th day of gestation), the total consumption of Ni at concentrations of 500 and 1000 ppm was estimated to be 1280 and 2560 mg/kg body mass, respectively.

Only the higher consumption of Ni caused any toxic response. Only 7 of the 27 mice that received 1000 ppm of Ni in their water were pregnant. Fetuses from the surviving pregnancies had a 15% loss of mass ($P = 0.009$). The decline in pregnancy rate and fetal mass appeared to be accompanied by maternal toxicity, as seen in the loss of mass in non-pregnant bred mice from the same high-dose group (Table 3). Manifestations of anomalies due to Ni consumption were not seen.

In the mouse, the LD_{50} to a single IP or oral dose of nickel acetate is 32 or 420 mg/kg body mass, respectively (Nickel, 1975). In this ratio the oral route is 13 times less effective for producing Ni toxicity. Lu et al. (1979)

observed a wide range and high incidence of terata in mice fetuses administered 4.6 mg of $NiCl_2$/kg body mass IP once between the 7th and 11th day of gestation. On the basis of the ratio of effectiveness of an oral vs. an injected dose, the 4.6-mg IP dose used by Lu would be equivalent to a 60-mg oral dose.

In our study, we could not induce the level of fetal response, i.e., the wide range and incidence of terata, seen by Lu after we administered Ni in 160 mg/kg orally daily (1000 ppm in drinking water). Only decreased fetal body mass was observed. Any higher dose was so toxic for both dam and conceptuses that the frequency did not continue. Therefore, our 160-mg/kg dose is the closest response to Lu's 4.6 mg/kg IP that we could achieve. As the calculated oral equivalent of 4.6 mg/kg IP is 60 mg/kg, our 1000-ppm dose is 2.7 times less effective. Therefore, such calculations do not adequately account for the observed differences.

The variation in results may depend on some other factor in the route of administration which decreases the effectiveness of low but continuous oral intake compared to high single parenteral route. It appears, therefore, that the fetal effects of Ni poisoning may be overestimated by experiments using parenteral methods.

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